IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : John Bertin
Serial No. :
Filed : HEREWITH
Title : NOVEL MOLECULES OF THE CARD-RELATED PROTEIN FAMILY AND USES THEREOF

Art Unit :
Examiner :

Commissioner for Patents
Washington, D.C. 20231

PRELIMINARY AMENDMENT

Prior to examination, please amend the application as follows:

In the specification:

Replace the paragraph beginning at page 1, line 8, with the following rewritten paragraph:

-- This application is a divisional of application serial number 09/099,041, filed June 17, 1998, which is a continuation-in-part of application serial number 09/019,942, filed February 6, 1998. --

Replace the paragraph beginning at page 3, line 24, with the following rewritten paragraph:

Two forms of CARD-4 exist in the cell, a long form, CARD-4L, and a short form, CARD-4S. The cDNA of CARD-4L described below (SEQ ID NO:7) has a 2859 nucleotide open reading frame (nucleotides 245-3103 of SEQ ID NO:7; SEQ ID NO:9) which encodes a 953 amino acid protein (SEQ ID NO:8). CARD-4L protein possesses a CARD domain (amino acids 15-114 of SEQ ID NO:8; SEQ ID NO:10). CARD-4L is also predicted to have a

CERTIFICATE OF MAILING BY EXPRESS MAIL

Express Mail Label No. EL932079165US

I hereby certify under 37 CFR §1.10 that this correspondence is being deposited with the United States Postal Service as Express Mail Post Office to Addressee with sufficient postage on the date indicated below and is addressed to the Commissioner for Patents, Washington, D.C. 20231.

January 22, 2002
Date of Deposit

Signature

Leroy Jenkins
Typed or Printed Name of Person Signing Certificate

nucleotide binding domain which extends from about amino acid 198 to about amino acid 397 of SEQ ID NO:8; SEQ ID NO:11, a Walker Box "A", which extends from about amino acid 202 to about amino acid 209 of SEQ ID NO:8; SEQ ID NO:12, a Walker Box "B", which extends from about amino acid 280 to about amino acid 284, of SEQ ID NO:8; SEQ ID NO:13, a kinase 3a subdomain, which extends from about amino acid 327 to about amino acid 338 of SEQ ID NO:8; SEQ ID NO:14, and ten Leucine-rich repeats which extend from about amino acid 674 to about amino acid 950 of SEQ ID NO:8. The first Leucine-rich repeat extends from about amino acid 674 to about amino acid 701 of SEQ ID NO:8; SEQ ID NO:15. The second Leucine-rich repeat extends from about amino acid 702 to about amino acid 727 of SEQ ID NO:8; SEQ ID NO:16. The third Leucine-rich repeat extends from about amino acid 728 to about amino acid 754 of SEQ ID NO:8; SEQ ID NO:17. The fourth Leucine-rich repeat extends from about amino acid 755 to about amino acid 782 of SEQ ID NO:8; SEQ ID NO:18. The fifth Leucine-rich repeat extends from about amino acid 783 to about amino acid 810 of SEQ ID NO:8; SEQ ID NO:19. The sixth Leucine-rich repeat extends from about amino acid 811 to about amino acid 838 of SEQ ID NO:8; SEQ ID NO:20. The seventh Leucine-rich repeat extends from about amino acid 839 to about amino acid 866 of SEQ ID NO:8; SEQ ID NO:21. The eighth Leucine-rich repeat extends from about amino acid 867 to about amino acid 894 of SEQ ID NO:8; SEQ ID NO:22. The ninth Leucine-rich repeat extends from about amino acid 895 to about amino acid 922 of SEQ ID NO:8; SEQ ID NO:23 and the tenth leucine-rich repeat extends from about amino acid 923 to about amino acid 950 of SEQ ID NO:8; SEQ ID NO:24.

Replace the paragraph beginning at page 7, line 16, with the following rewritten paragraph:

The invention features a nucleic acid molecule which is at least 45% (or 55%, 65%, 75%, 85%, 95%, or 98%) identical to the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:7, SEQ ID NO:9, SEQ ID:25, SEQ ID NO:27, the nucleotide sequence of the cDNA insert of the plasmid deposited with ATCC as Accession Number (the "cDNA of ATCC 203037"), the nucleotide sequence of the cDNA insert of the plasmid deposited with ATCC as Accession Number (the "cDNA of ATCC 203035"), the nucleotide sequence of the cDNA insert

of the plasmid deposited with ATCC as Accession Number (the "cDNA of ATCC 203036"), or a complement thereof.

Replace the paragraph beginning at page 7, line 28, with the following rewritten paragraph:

The invention features a nucleic acid molecule which includes a fragment of at least 150 (300, 325, 350, 375, 400, 425, 450, 500, 550, 600, 650, 700, 800, 900, 1000, 1300, 1600 or 1931) nucleotides of the nucleotide sequence shown in SEQ ID NO:1, or SEQ ID NO:3, or the nucleotide sequence of the cDNA ATCC 203037, or a complement thereof.

Replace the paragraph beginning at page 7, line 33, with the following rewritten paragraph:

The invention also features a nucleic acid molecule which includes a fragment of at least 150 (350, 400, 450, 500, 550, 600, 650, 700, 800, 900, 1000, 1300, 1600, 1900, 2100, 2400, 2700, 3000, or 3382) nucleotides of the nucleotide sequence shown in SEQ ID NO:7, or SEQ ID NO:9, or the nucleotide sequence of the cDNA ATCC 203035, or a complement thereof.

Replace the paragraph beginning at page 8, line 6, with the following rewritten paragraph:

Also within the invention is a nucleic acid molecule which includes a fragment of at least 150 (350, 400, 450, 500, 550, 600, 650, 700, 800, 900, 1000, 1300, 1600, 1900, 2100, 2400, 2700, and 3080) nucleotides of the nucleotide sequence shown in SEQ ID NO:25, or SEQ ID NO:27, or the nucleotide sequence of the cDNA ATCC 203036, or a complement thereof.

Replace the paragraph beginning at page 8, line 13, with the following rewritten paragraph:

The invention features a nucleic acid molecule which includes a nucleotide sequence encoding a protein having an amino acid sequence that is at least 45% (or 55%, 65%, 75%, 85%, 95%, or 98%) identical to the amino acid sequence of SEQ ID NO:2, SEQ ID NO:8, SEQ ID NO: 26 or the amino acid sequence encoded by the cDNA of ATCC 203037, the amino acid

sequence encoded by the cDNA of ATCC 203035, or the amino acid sequence encoded by the cDNA of ATCC 203036.

Replace the paragraph beginning at page 8, line 21, with the following rewritten paragraph:

In a preferred embodiment, a CARD-3 nucleic acid molecule has the nucleotide sequence shown in SEQ ID NO:1, or SEQ ID NO:3, or the nucleotide sequence of the cDNA of ATCC 203037. In another preferred embodiment, a CARD-4L nucleic acid molecule has the nucleotide sequence shown in SEQ ID NO:7, or SEQ ID NO:9, or the nucleotide sequence of the cDNA of ATCC 203035. In yet another preferred embodiment, a CARD-4S nucleic acid molecule has the nucleotide sequence shown in SEQ ID NO:25, or SEQ ID NO:27, or the nucleotide sequence of the cDNA of ATCC 203036.

Replace the paragraph beginning at page 8, line 31, with the following rewritten paragraph:

Also within the invention is a nucleic acid molecule which encodes a fragment of a polypeptide having the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:8 or SEQ ID NO:26, the fragment including at least 15 (25, 30, 50, 100, 150, 300, 400 or 540, 600, 700, 800, 953) contiguous amino acids of SEQ ID NO:2 or SEQ ID NO:8 or SEQ ID NO:26 or the polypeptide encoded by the cDNA of ATCC Accession Number 203037, or the polypeptide encoded by the cDNA of ATCC Accession Number 203035, or the polypeptide encoded by the cDNA of ATCC Accession Number 203036.

Replace the paragraph beginning at page 9, line 7, with the following rewritten paragraph:

The invention includes a nucleic acid molecule which encodes a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2 or an amino acid sequence encoded by the cDNA of ATCC Accession Number 203037, wherein the nucleic acid molecule hybridizes to a nucleic acid molecule comprising SEQ ID NO:1 or SEQ ID NO:3 under stringent conditions. The invention also includes a nucleic acid molecule which

encodes a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:8 or an amino acid sequence encoded by the cDNA of ATCC Accession Number 203035, wherein the nucleic acid molecule hybridizes to a nucleic acid molecule comprising SEQ ID NO:7 or SEQ ID NO:9 under stringent conditions.

Replace the paragraph beginning at page 9, line 21, with the following rewritten paragraph:

The invention also includes a nucleic acid molecule which encodes a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:26 or an amino acid sequence encoded by the cDNA of ATCC Accession Number 203036, wherein the nucleic acid molecule hybridizes to a nucleic acid molecule comprising SEQ ID NO:25 or SEQ ID NO:27 under stringent conditions.

Replace the paragraph beginning at page 11, line 11, with the following rewritten paragraph:

Also within the invention are: an isolated CARD-3 protein which is encoded by a nucleic acid molecule having a nucleotide sequence that is at least about 65%, preferably 75%, 85%, or 95% identical to SEQ ID NO:3 or the cDNA of ATCC 203037; an isolated CARD-3 protein which is encoded by a nucleic acid molecule having a nucleotide sequence at least about 65% preferably 75%, 85%, or 95% identical to the kinase domain encoding portion of SEQ ID NO:1 (e.g., about nucleotides 213 to 1113 of SEQ ID NO:1); an isolated CARD-3 protein which is encoded by a nucleic acid molecule having a nucleotide sequence at least about 65% preferably 75%, 85%, or 95% identical the linker domain encoding portion of SEQ ID NO:1 (e.g., about nucleotides 1114 to 1506 of SEQ ID NO:1); and an isolated CARD-3 protein which is encoded by a nucleic acid molecule having a nucleotide sequence at least about 65% preferably 75%, 85%, or 95% identical the CARD domain encoding portion of SEQ ID NO:1 (e.g., about nucleotides 1507 to 1833 of SEQ ID NO:1); and an isolated CARD-3 protein which is encoded by a nucleic acid molecule having a nucleotide sequence which hybridizes under stringent hybridization conditions to a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:3 or the non-coding strand of the cDNA of ATCC 203037.

Replace the paragraph beginning at page 11, line 34, with the following rewritten paragarph:

Also within the invention are: an isolated CARD-4L protein which is encoded by a nucleic acid molecule having a nucleotide sequence that is at least about 65%, preferably 75%, 85%, or 95% identical to SEQ ID NO:9 or the cDNA of ATCC 203035; an isolated CARD-4L protein which is encoded by a nucleic acid molecule having a nucleotide sequence at least about 65% preferably 75%, 85%, or 95% identical the CARD domain encoding portion of SEQ ID NO:7 (e.g., about nucleotides 287 to 586 of SEQ ID NO:7); an isolated CARD-4L protein which is encoded by a nucleic acid molecule having a nucleotide sequence at least about 65% preferably 75%, 85%, or 95% identical the nucleotide binding domain encoding portion of SEQ ID NO:7 (e.g., about nucleotides 836 to 1436 of SEQ ID NO:7); an isolated CARD-4L protein which is encoded by a nucleic acid molecule having a kinase 3a subdomain at least about 65% preferably 75%, 85%, or 95% identical the nucleotide binding domain encoding portion of SEQ ID NO:7 (e.g., about nucleotides 1223 to 1258 of SEQ ID NO:7); an isolated CARD-4L protein which is encoded by a nucleic acid molecule having a nucleotide sequence at least about 65% preferably 75%, 85%, or 95% identical the Leucine-rich repeats encoding portion of SEQ ID NO:7 (e.g., about nucleotides 2264 to 2347 of SEQ ID NO:7; about nucleotides 2348 to 2425 of SEQ ID NO:7; about nucleotides 2426 to 2506 of SEQ ID NO:7; about nucleotides 2507 to 2590 of SEQ ID NO:7; about nucleotides 2591 to 2674 of SEQ ID NO:7; about nucleotides 2675 to 2758 of SEQ ID NO:7; about nucleotides 2759 to 2842 of SEQ ID NO:7; about nucleotides 2843 to 2926 of SEQ ID NO:7; about nucleotides 2927 to 3010 of SEQ ID NO:7; about nucleotides 3011 to 3094 of SEQ ID NO:7; and an isolated CARD-4L protein which is encoded by a nucleic acid molecule having a nucleotide sequence which hybridizes under stringent hybridization conditions to a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:9 or the non-coding strand of the cDNA of ATCC 203035.

Replace the paragraph beginning at page 13, line 1, with the following rewritten paragraph:

Also within the invention are: an isolated CARD-4S protein which is encoded by a nucleic acid molecule having a nucleotide sequence that is at least about 65%, preferably 75%, 85%, or 95% identical to SEQ ID NO:27 or the cDNA of ATCC 203036; an isolated CARD-3 protein which is encoded by a nucleic acid molecule having a nucleotide sequence at least about 65% preferably 75%, 85%, or 95% identical the CARD domain encoding portion of SEQ ID NO:25 (e.g., about nucleotides 1 to 222 of SEQ ID NO:25); an isolated CARD-3 protein which is encoded by a nucleic acid molecule having a nucleotide sequence at least about 65% preferably 75%, 85%, or 95% identical the P-Loop encoding portion of SEQ ID NO:25 (e.g., about nucleotides 485 to 510 of SEQ ID NO:25).

Replace the paragraph beginning at page 13, line 14, with the following rewritten paragraph:

Also within the invention is a polypeptide which is a naturally occurring allelic variant of a polypeptide that includes the amino acid sequence of SEQ ID NO:2 or an amino acid sequence encoded by the cDNA insert of the plasmid deposited with ATCC as Accession Number 203037, wherein the polypeptide is encoded by a nucleic acid molecule which hybridizes to a nucleic acid molecule comprising SEQ ID NO:1 or SEQ ID NO:3 under stringent conditions.

Replace the paragraph beginning at page 13, line 22, with the following rewritten paragraph:

Also within the invention is a polypeptide which is a naturally occurring allelic variant of a polypeptide that includes the amino acid sequence of SEQ ID NO:8 or an amino acid sequence encoded by the cDNA insert of the plasmid deposited with ATCC as Accession Number 203035, wherein the polypeptide is encoded by a nucleic acid molecule which hybridizes to a nucleic acid molecule comprising SEQ ID NO:7 or SEQ ID NO:9 under stringent conditions.

Replace the paragraph beginning at page 13, line 30, with the following rewritten paragraph:

Also within the invention is a polypeptide which is a naturally occurring allelic variant of a polypeptide that includes the amino acid sequence of SEQ ID NO:26 or an amino acid sequence encoded by the cDNA insert of the plasmid deposited with ATCC as Accession Number 203036, wherein the polypeptide is encoded by a nucleic acid molecule which hybridizes to a nucleic acid molecule comprising SEQ ID NO:25 or SEQ ID NO:27 under stringent conditions.

Replace the paragraph beginning at page 14, line 4, with the following rewritten paragraph:

Another embodiment of the invention features CARD-3 or CARD-4L/S nucleic acid molecules which specifically detect CARD-3 or CARD-4L/S nucleic acid molecules, relative to nucleic acid molecules encoding other members of the CARD superfamily. For example, in one embodiment, a CARD-4L nucleic acid molecule hybridizes under stringent conditions to a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:7, SEQ ID NO:9, or the cDNA of ATCC 203035, or a complement thereof. In another embodiment, the CARD-4L nucleic acid molecule is at least 300 (350, 400, 450, 500, 550, 600, 650, 700, 800, 900, 1000, 1300, 1600, 1900, 2100, 2400, 2700, 3000, or 3382) nucleotides in length and hybridizes under stringent conditions to a nucleic acid molecule comprising the nucleotide sequence shown in SEQ ID NO:7, SEQ ID NO:9, the cDNA of ATCC 203035, or a complement thereof. In another embodiment, an isolated CARD-4L nucleic acid molecule comprises nucleotides 287 to 586 of SEQ ID NO:7, encoding the CARD domain of CARD-4L, or a complement thereof. In yet another embodiment, the invention provides an isolated nucleic acid molecule which is antisense to the coding strand of a CARD-4L nucleic acid.

Replace the paragraph beginning at page 17, line 21, with the following rewritten paragraph:

Figures 3A-3B depict the cDNA sequence (SEQ ID NO:7) of CARD-4L. The open reading frame of SEQ ID NO:7 extends from nucleotide 245 to nucleotide 3103 (SEQ ID NO:9).

Replace the paragraph beginning at page 17, line 26, with the following rewritten paragraph:

Figure 5 depicts the partial cDNA sequence (SEQ ID NO:25) of CARD-4S. The open reading frame of CARD-4S (SEQ ID NO:25) extends from nucleotide 1 to nucleotide 1470 (SEQ ID NO:27).

Replace the paragraph beginning at page 17, line 33, with the following rewritten paragraph:

Figure 7 depicts an alignment of the CARD domains of CARD-4 (SEQ ID NO:10), CARD-3 (SEQ ID NO:6), ARC-CARD (SEQ ID NO:31), cIAP1-CARD (SEQ ID NO:32), and cIAP2-CARD (SEQ ID NO:33).

Replace the paragraph beginning at page 18, line 9, with the following rewritten paragraph:

The present invention is based on the discovery of a cDNA molecule encoding human CARD-3 and human CARD-4 proteins. A nucleotide sequence encoding a human CARD-3 protein is shown in Figure 1 (SEQ ID NO:1; SEQ ID NO:3 includes the open reading frame only). A predicted amino acid sequence of CARD-3 protein is also shown in Figure 2 (SEQ ID NO: 2). CARD-4 has two forms, a long form, CARD-4L, and a short form, CARD-4S. A nucleotide sequence encoding a human CARD-4L protein is shown in Figures 3A-3B (SEQ ID NO:7; SEQ ID NO:9 includes the open reading frame only). A predicted amino acid sequence of CARD-4L protein is also shown in Figure 4 (SEQ ID NO:8). A nucleotide sequence encoding a partial human CARD-4S protein is shown in Figure 5 (SEQ ID NO:25; SEQ ID NO:27 includes the open reading frame only). A predicted amino acid sequence of CARD-4S protein is also shown in Figure 6 (SEQ ID NO:26).

Replace the paragraph beginning at page 18, line 25, with the following rewritten paragraph:

The human CARD-3 cDNA of Figure 1 (SEQ ID NO:1), which is approximately 1931 nucleotides long including untranslated regions, encodes a protein amino acid having a molecular weight of approximately 61 kDa (excluding post-translational modifications). A plasmid containing a cDNA encoding human CARD-3 (pXE17A) was deposited with the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA on May 14, 1998, and assigned Accession Number 203037. This deposit will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. This deposit was made merely as a convenience for those of skill in the art and is not an admission that a deposit is required under 35 U.S.C. §112.

Replace the paragraph beginning at page 19, line 5, with the following rewritten paragraph:

The human CARD-4L cDNA of Figures 3A-3B (SEQ ID NO:7), which is approximately 3382 nucleotides long including untranslated regions, encodes a protein amino acid having a molecular weight of approximately 108 kDa (excluding post-translational modifications). A plasmid containing a cDNA encoding human CARD-4L (pC4L1) was deposited with the American Type Culture Collection (ATCC), 1801 University Boulevard, Manassas, VA on May 14, 1998, and assigned Accession Number 203035. This deposit will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. This deposit was made merely as a convenience for those of skill in the art and is not an admission that a deposit is required under 35 U.S.C §112.

Replace the paragraph beginning at page 19, line 19, with the following rewritten paragraph:

The human partial CARD-4S cDNA of Figure 5 (SEQ ID NO:25), which is approximately 3080 nucleotides long including untranslated regions. A plasmid containing a cDNA encoding human CARD-4S (pDB33E) was deposited with the American Type Culture

Collection (ATCC), 1801 University Boulevard, Manassas, VA on May 14, 1998, and assigned Accession Number 203036. This deposit will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. This deposit was made merely as a convenience for those of skill in the art and is not an admission that a deposit is required under 35 U.S.C. §112.

Replace the paragraph beginning at page 19, line 31, with the following rewritten paragraph:

A region of human CARD-4L protein (SEQ ID NO:8) bears some similarity to a CARD domain of CARD-3 (SEQ ID NO:6), ARC-CARD (SEQ ID NO:31), cIAP1-CARD (SEQ ID NO:32), and cIAP2-CARD (SEQ ID NO:33). This comparison is depicted in Figure 7.

Replace the paragraph beginning at page 20, line 22, with the following rewritten paragarph:

Preferred CARD-3 or CARD-4L or CARD-4S polypeptides of the present invention have an amino acid sequence sufficiently identical to the CARD domain consensus amino acid sequence of SEQ ID NO:6, SEQ ID NO:10, SEQ ID NO:28, respectively. The CARD-3 polypeptide also has an amino acid sequence sufficiently identical to the kinase domain consensus sequence of SEQ ID NO:4, and an amino acid sequence that is sufficiently identical to the linker domain of SEQ ID NO:5. The CARD-4L polypeptide has an amino acid sequence sufficiently identical to the nucleotide binding domain of SEQ ID NO:11, an amino acid sequence sufficiently identical to the Walker Box "A" of SEQ ID NO:12 or Walker Box "B" of SEQ ID NO:13, or an amino acid sequence sufficiently identical to the kinase 3a subdomain of SEQ ID NO:14, or an amino acid sequence sufficiently identical to the Leucine-rich repeats of SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO: 19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO: 22, SEQ ID NO: 23, and SEQ ID NO:24. As used herein, the term "sufficiently identical" refers to a first amino acid or nucleotide sequence which contains a sufficient or minimum number of identical or equivalent (e.g., an amino acid residue which has a similar side chain) amino acid residues or nucleotides to a second amino acid or

nucleotide sequence such that the first and second amino acid or nucleotide sequences have a common structural domain and/or common functional activity. For example, amino acid or nucleotide sequences which contain a common structural domain having about 65% identity, preferably 75% identity, more preferably 85%, 95%, or 98% identity are defined herein as sufficiently identical.

Replace the paragraph beginning at page 23, line 7, with the following rewritten paragraph:

A nucleic acid molecule of the present invention, e.g., a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO: 25, SEQ ID NO: 27, the cDNA of ATCC 203037, the cDNA of ATCC 203035, or the cDNA of ATCC 203036, or a complement of any of these nucleotide sequences, can be isolated using standard molecular biology techniques and the sequence information provided herein. Using all or portion of the nucleic acid sequences of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO: 25, SEQ ID NO: 27, the cDNA of ATCC 203037, the cDNA of ATCC 203035, or the cDNA of ATCC 203036 as a hybridization probe, CARD-3 or CARD-4L/S nucleic acid molecules can be isolated using standard hybridization and cloning techniques (e.g., as described in Sambrook et al., eds., *Molecular Cloning: A Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory,* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989).

Replace the paragraph beginning at page 23, line 33, with the following rewritten paragraph:

In another preferred embodiment, an isolated nucleic acid molecule of the invention comprises a nucleic acid molecule which is a complement of the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO: 25, SEQ ID NO: 27, the cDNA of ATCC 203037, the cDNA of ATCC 203035, the cDNA of ATCC 203036, or a portion thereof. A nucleic acid molecule which is complementary to a given nucleotide sequence is one which is sufficiently complementary to the given nucleotide sequence that it can hybridize to the given nucleotide sequence thereby forming a stable duplex.

Replace the paragraph beginning at page 24, line 10, with the following rewritten paragraph:

Moreover, the nucleic acid molecule of the invention can comprise only a portion of a nucleic acid sequence encoding CARD-3 or CARD-4L/S, for example, a fragment which can be used as a probe or primer or a fragment encoding a biologically active portion of CARD-3 or CARD-4L/S. The nucleotide sequence determined from the cloning of the human CARD-3 or CARD-4L/S gene allows for the generation of probes and primers designed for use in identifying and/or cloning CARD-3 or CARD-4L/S homologues in other cell types, e.g., from other tissues, as well as CARD-3 or CARD-4L/S homologues from other mammals. The probe/primer typically comprises substantially purified oligonucleotide. The oligonucleotide typically comprises a region of nucleotide sequence that hybridizes under stringent conditions to at least about 12, preferably about 25, more preferably about 50, 75, 100, 125, 150, 175, 200, 250, 300, 350 or 400 consecutive nucleotides of the sense or anti-sense sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:7, SEQ ID NO:9, the cDNA of ATCC 203037, the cDNA of ATCC 203035, or of a naturally occurring mutant of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:7, SEQ ID NO:9, the cDNA of ATCC 203037, or the cDNA of ATCC 203035.

Replace the paragraph beginning at page 25, line 11, with the following rewritten paragraph:

A nucleic acid fragment encoding a "biologically active portion of CARD-3 or CARD-4L/S" can be prepared by isolating a portion of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:7, SEQ ID NO:9, or the nucleotide sequence of the cDNA of ATCC 203037, or the nucleotide sequence of the cDNA of ATCC 203035 which encodes a polypeptide having a CARD-3 or CARD-4L/S biological activity, expressing the encoded portion of CARD-3 or CARD-4L/S protein (e.g., by recombinant expression *in vitro*) and assessing the activity of the encoded portion of CARD-3 or CARD-4L/S. For example, a nucleic acid fragment encoding a biologically active portion of CARD-3 or CARD-4L/S includes a CARD domain, e.g., SEQ ID NO:6 and SEQ ID NO:10 or SEQ ID NO:28.

Replace the paragraph beginning at page 25, line 24, with the following rewritten paragraph:

The invention further encompasses nucleic acid molecules that differ from the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:25, SEQ ID NO:27, the cDNA of ATCC 203037, or the cDNA of ATCC 203035, or the cDNA of ATCC 203036 due to degeneracy of the genetic code and thus encode the same CARD-3 or CARD-4L/S protein as that encoded by the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:25, SEQ ID NO:27, the cDNA of ATCC 203037, the cDNA of ATCC 203035, or the cDNA of ATCC 203036.

Replace the paragraph beginning at page 25, line 34, with the following rewritten paragraph:

In addition to the human CARD-3 or CARD-4L/S nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:25, SEQ ID NO:27, the cDNA of ATCC 203037, the cDNA of ATCC 203035, or the cDNA of ATCC 203036, it will be appreciated by those skilled in the art that DNA sequence polymorphisms that lead to changes in the amino acid sequences of CARD-3 or CARD-4L/S may exist within a population (e.g., the human population). Such genetic polymorphism in the CARD-3 or CARD-4L/S gene may exist among individuals within a population due to natural allelic variation. As used herein, the terms "gene" and "recombinant gene" refer to nucleic acid molecules comprising an open reading frame encoding a CARD-3 or CARD-4L/S protein, preferably a mammalian CARD-3 or CARD-4L/S protein. Such natural allelic variations can typically result in 1-5% variance in the nucleotide sequence of the CARD-3 or CARD-4L/S gene. Any and all such nucleotide variations and resulting amino acid polymorphisms in CARD-3 or CARD-4L/S that are the result of natural allelic variation and that do not alter the functional activity of CARD-3 or CARD-4L/S are intended to be within the scope of the invention.

Replace the paragraph beginning at page 26, line 34, with the following rewritten paragraph:

Accordingly, in another embodiment, an isolated nucleic acid molecule of the invention is at least 300 (325, 350, 375, 400, 425, 450, 500, 550, 600, 650, 700, 800, 900, 1000, 1300, 1600 or 1931) nucleotides in length and hybridizes under stringent conditions to the nucleic acid molecule comprising the nucleotide sequence, preferably the coding sequence, of SEQ ID NO:1, SEQ ID NO:3, or the cDNA of ATCC 203037. In yet another embodiment, an isolated nucleic acid molecule of the invention is at least 300 (325, 350, 375, 400, 425, 450, 500, 550, 600, 650, 700, 800, 900, 1000, or 1300, 1640, 1900, 2200, 2500, 2800, 3100, or 3382) nucleotides in length and hybridizes under stringent conditions to the nucleic acid molecule comprising the nucleotide sequence, preferably the coding sequence, of SEQ ID NO:7, SEQ ID NO:9, the cDNA of ATCC 203035.

Replace the paragraph beginning at page 27, line 15, with the following rewritten paragraph:

As used herein, the term "hybridizes under stringent conditions" is intended to describe conditions for hybridization and washing under which nucleotide sequences at least 60% (65%, 70%, preferably 75%) identical to each other typically remain hybridized to each other. Such stringent conditions are known to those skilled in the art and can be found in *Current Protocols in Molecular Biology*, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. A preferred, non-limiting example of stringent hybridization conditions are hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45EC, followed by one or more washes in 0.2 X SSC, 0.1% SDS at 50-65EC. Preferably, an isolated nucleic acid molecule of the invention that hybridizes under stringent conditions to the sequence of SEQ ID NO:1, SEQ ID NO:3, the cDNA of ATCC 203037 corresponds to a naturally-occurring nucleic acid molecule. As used herein, a "naturally-occurring" nucleic acid molecule refers to an RNA or DNA molecule having a nucleotide sequence that occurs in nature (e.g., encodes a natural protein).

Replace the paragraph beginning at page 27, line 34, with the following rewritten paragraph:

In addition to naturally-occurring allelic variants of the CARD-3 or CARD-4L/S sequence that may exist in the population, the skilled artisan will further appreciate that changes can be introduced by mutation into the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:25, SEQ ID NO:27, the cDNA of ATCC 203037, the cDNA of ATCC 203035, or the cDNA of ATCC 203036, thereby leading to changes in the amino acid sequence of the encoded CARD-3 or CARD-4L/S protein, without altering the functional ability of the CARD-3 or CARD-4L/S protein. For example, one can make nucleotide substitutions leading to amino acid substitutions at "non-essential" amino acid residues. A "non-essential" amino acid residue is a residue that can be altered from the wild-type sequence of CARD-3 or CARD-4L/S (e.g., the sequence of SEQ ID NO:2 or SEQ ID NO:8 or SEQ ID NO:26) without altering the biological activity, whereas an "essential" amino acid residue is required for biological activity. For example, amino acid residues that are conserved among the CARD-3 or CARD-4L/S proteins of various species are predicted to be particularly unamenable to alteration.

Replace the paragraph beginning at page 28, line 32, with the following rewritten paragraph:

Accordingly, another aspect of the invention pertains to nucleic acid molecules encoding CARD-3 or CARD-4L/S proteins that contain changes in amino acid residues that are not essential for activity. Such CARD-3 or CARD-4L/S proteins differ in amino acid sequence from SEQ ID NO:2 or SEQ ID NO: 8 or SEQ ID NO:26 and yet retain biological activity. In one embodiment, the isolated nucleic acid molecule includes a nucleotide sequence encoding a protein that includes an amino acid sequence that is at least about 45% identical, 65%, 75%, 85%, 95%, or 98% identical to the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:8 or SEQ ID NO:26.

Replace the paragraph beginning at page 29, line 10, with the following rewritten paragraph:

An isolated nucleic acid molecule encoding a CARD-3 or CARD-4L/S protein having a sequence which differs from that of SEQ ID NO:2 or SEQ ID NO:8 or SEQ ID NO:26, respectively, can be created by introducing one or more nucleotide substitutions, additions or deletions into the nucleotide sequence of CARD-3 (SEQ ID NO:1, SEQ ID NO:3, the cDNA of ATCC 203037) or CARD-4L (SEQ ID NO:7, SEQ ID NO:9, the cDNA of ATCC 203035), or CARD-4S (SEQ ID NO:25, SEQ ID NO:27, the cDNA of ATCC 203036) such that one or more amino acid substitutions, additions or deletions are introduced into the encoded protein. Mutations can be introduced by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis. Preferably, conservative amino acid substitutions are made at one or more predicted non-essential amino acid residues. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Thus, a predicted nonessential amino acid residue in CARD-3 or CARD-4L/S is preferably replaced with another amino acid residue from the same side chain family. Alternatively, mutations can be introduced randomly along all or part of a CARD-3 or CARD-4L/S coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for CARD-3 or CARD-4L/S biological activity to identify mutants that retain activity. Following mutagenesis, the encoded protein can be expressed recombinantly and the activity of the protein can be determined.

Replace the paragraph beginning at page 31, line 4, with the following rewritten paragraph:

Given the coding strand sequences encoding CARD-3 or CARD-4L/S disclosed herein (e.g., SEQ ID NO:1 or SEQ ID NO:3 or SEQ ID NO:7 or SEQ ID NO:9 or SEQ ID NO:25 or SEQ ID NO:27), antisense nucleic acids of the invention can be designed according to the rules of Watson and Crick base pairing. The antisense nucleic acid molecule can be complementary to the entire coding region of CARD-3 or CARD-4L/S mRNA, but more preferably is an oligonucleotide which is antisense to only a portion of the coding or noncoding region of CARD-3 or CARD-4L/S mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding the translation start site of CARD-3 mRNA, e.g., an oligonucleotide having the sequence CCCTGGTACTTGCCCCTCCGGTAG (SEQ ID NO:34) or CCTGGTACTTGCCCCTCC (SEQ ID NO:35) or of the CARD-4L mRNA e.g., TCGTTAAGCCCTTGAAGACAGTG (SEQ ID NO:36) and TCGTTAGCCCTTGAAGACCAGTGAGTGTAG (SEQ ID NO:37). An antisense oligonucleotide can be, for example, about 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 nucleotides in length. An antisense nucleic acid of the invention can be constructed using chemical synthesis and enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid (e.g., an antisense oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, e.g., phosphorothioate derivatives and acridine substituted nucleotides can be used. Examples of modified nucleotides which can be used to generate the antisense nucleic acid include 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-

thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine. Alternatively, the antisense nucleic acid can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (i.e., RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest, described further in the following subsection).

Replace the paragraph beginning at page 38, line 9, with the following rewritten paragraph:

Biologically active portions of a CARD-3 or CARD-4L/S protein include peptides comprising amino acid sequences sufficiently identical to or derived from the amino acid sequence of the CARD-3 or CARD-4L/S protein (e.g., the amino acid sequence shown in SEQ ID NO:2 or SEQ ID NO:8 or SEQ ID NO:26), which include less amino acids than the full length CARD-3 or CARD-4L/S proteins, and exhibit at least one activity of a CARD-3 or CARD-4L/S protein. Typically, biologically active portions comprise a domain or motif with at least one activity of the CARD-3 or CARD-4L/S protein. A biologically active portion of a CARD-3 or CARD-4L/S protein can be a polypeptide which is, for example, 10, 25, 50, 100 or more amino acids in length. Preferred biologically active polypeptides include one or more identified CARD-3 or CARD-4L/S structural domains, e.g., the CARD domain (SEQ ID NO:6 or SEQ ID NO:10 or SEQ ID NO:28).

Replace the paragraph beginning at page 38, line 30, with the following rewritten paragraph:

Preferred CARD-3 or CARD-4L/S protein has the amino acid sequence shown of SEQ ID NO:2 or SEQ ID NO:8 or SEQ ID NO:26, respectively. Other useful CARD-3 or CARD-4L/S proteins are substantially identical to SEQ ID NO:2 or SEQ ID NO:8 or SEQ ID NO:26, respectively, and retain the functional activity of the protein of SEQ ID NO:2 or SEQ ID NO:8 or SEQ ID NO:26, yet differ in amino acid sequence due to natural allelic variation or mutagenesis. CARD-3 and CARD-4L/S may be involved in activating caspases in the apoptotic

pathway. Accordingly, a useful CARD-3 or CARD-4L/S protein is a protein which includes an amino acid sequence at least about 45%, preferably 55%, 65%, 75%, 85%, 95%, or 99% identical to the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:8 or SEQ ID NO:26 and retains the functional activity of the CARD-3 or CARD-4L/S proteins of SEQ ID NO:2 or SEQ ID NO:8 or SEQ ID NO:26. In other instances, the CARD-3 or CARD-4L/S protein is a protein having an amino acid sequence 55%, 65%, 75%, 85%, 95%, or 98% identical to the CARD-3 or CARD-4L CARD domain (SEQ ID NO:6, SEQ ID NO:10 and SEQ ID NO:28). In a preferred embodiment, the CARD-3 or CARD-4L/S protein retains a functional activity of the CARD-3 or CARD-4L/S protein of SEQ ID NO:2, SEQ ID NO:8 or SEQ ID NO:26.

Replace the paragraph beginning at page 60, line 6, with the following rewritten paragraph:

A transgenic animal of the invention can be created by introducing CARD-3 or CARD-4L/S-encoding nucleic acid into the male pronuclei of a fertilized oocyte, e.g., by microinjection, retroviral infection, and allowing the oocyte to develop in a pseudopregnant female foster animal. The CARD-3 or CARD-4L/S cDNA sequence e.g., that of (SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:25, SEQ ID NO:27, or the cDNA of ATCC 203037, or the cDNA of ATCC 203035, or the cDNA of ATCC 203036) can be introduced as a transgene into the genome of a non-human animal. Alternatively, a nonhuman homologue of the human CARD-3 or CARD-4L/S gene, such as a mouse CARD-3 or CARD-4L/S gene, can be isolated based on hybridization to the human CARD-3 or CARD-4L/S cDNA and used as a transgene. Intronic sequences and polyadenylation signals can also be included in the transgene to increase the efficiency of expression of the transgene. A tissue-specific regulatory sequence(s) can be operably linked to the CARD-3 or CARD-4L/S transgene to direct expression of CARD-3 or CARD-4L/S protein to particular cells. Methods for generating transgenic animals via embryo manipulation and microinjection, particularly animals such as mice, have become conventional in the art and are described, for example, in U.S. Patent Nos. 4,736,866 and 4,870,009, U.S. Patent No. 4,873,191 and in Hogan, *Manipulating the Mouse Embryo*, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1986). Similar methods are used for production of other transgenic animals.

Replace the paragraph beginning at page 104, line 9, with the following rewritten paragraph:

The human CARD-4L cDNA was isolated as described above (Figures 3A-3B; SEQ ID NO:7) and has a 2859 nucleotide open reading frame (nucleotides 245-3103 of SEQ ID NO:7; SEQ ID NO:9) which encodes a 953 amino acid protein (Figure 4; SEQ ID NO:8). CARD-4L protein has a predicted CARD domain (amino acids 15-114; SEQ ID NO:10). CARD-4L is also predicted to have a nucleotide binding domain which extends from about amino acid 198 to about amino acid 397 of SEQ ID NO:8; SEQ ID NO:11, a predicted Walker Box "A", which extends from about amino acid 202 to about amino acid 209 of SEQ ID NO:8; SEQ ID NO:12, a predicted Walker Box "B", which extends from about amino acid 280 to about amino acid 284, of SEQ ID NO:8; SEQ ID NO:13, a predicted kinase 3a subdomain, which extends from about amino acid 327 to about amino acid 338 of SEQ ID NO:8; SEQ ID NO:14, and ten predicted Leucine-rich repeats which extend from about amino acid 674 to about amino acid 950 of SEQ ID NO:8. The first Leucine-rich repeat is predicted to extend from about amino acid 674 to about amino acid 701 of SEQ ID NO:8; SEQ ID NO:15. The second Leucine-rich repeat is predicted to extend from about amino acid 702 to about amino acid 727 of SEQ ID NO:8; SEQ ID NO:16. The third Leucine-rich repeat is predicted to extend from about amino acid 728 to about amino acid 754 of SEQ ID NO:8; SEQ ID NO:17. The fourth Leucine-rich repeat is predicted to extend from about amino acid 755 to about amino acid 782 of SEQ ID NO:8; SEQ ID NO:18. The fifth Leucine-rich repeat is predicted to extend from about amino acid 783 to about amino acid 810 of SEQ ID NO:8; SEQ ID NO:19. The sixth Leucine-rich repeat is predicted to extend from about amino acid 811 to about amino acid 838 of SEQ ID NO:8; SEQ ID NO:20. The seventh Leucine-rich repeat is predicted to extend from about amino acid 839 to about amino acid 866 of SEQ ID NO:8; SEQ ID NO:21. The eighth Leucine-rich repeat is predicted to extend from about amino acid 867 to about amino acid 894 of SEQ ID NO:8; SEQ ID NO:22. The ninth Leucine-rich repeat is predicted to extend from about amino acid 895 to about amino acid 922 of SEQ ID NO:8; SEQ ID NO:23 and the tenth leucine-rich repeat is predicted to extend from about amino acid 923 to about amino acid 950 of SEQ ID NO:8; SEQ ID NO:24.

In the claims:

Cancel claims 1 to 23.

Add new claims 24-32 as follows.

-- 24. A purified antibody that specifically binds a polypeptide consisting of the amino acid sequence of SEQ ID NO:8.

25. A purified antibody that specifically binds to a polypeptide consisting of the amino acid sequence of amino acids 15-114 of SEQ ID NO:8 (CARD domain).

26. A purified antibody that specifically binds to a polypeptide consisting of the amino acid sequence of amino acids 198-397 of SEQ ID NO:8 (nucleotide binding domain).

27. A purified antibody that specifically binds to a polypeptide consisting of the amino acid sequence of amino acids 674-950 of SEQ ID NO:8 (leucine rich repeat domain).

28. The purified antibody of claim 24 wherein the antibody is a monoclonal antibody.

29. The purified antibody of claim 24 wherein the antibody is a chimeric antibody.

30. The purified antibody of claim 24 wherein the antibody is a humanized antibody.

31. The purified antibody of claim 24 wherein the antibody is detectably labeled.

32. The purified antibody of claim 31 wherein the detectable label is selected from the group consisting of: an enzyme, a prosthetic group, a fluorescent material, a luminescent material, a bioluminescent material, and a radioactive material.--

<u>In the drawings</u>:

Substitute the enclosed formal drawings for the informal drawings on file.

REMARKS

This amendment is made to correct the description of the drawings to correspond with the formal drawings being filed herewith, to insert the appropriate ATCC Deposit Numbers, and to correct SEQ ID NOS., in the specification where applicable. No new matter is introduced.

Attached is a marked-up version of the changes being made by the current amendment.

Applicant asks that all claims be examined. Please apply any other charges or credits to Deposit Account No. 06-1050.

Respectfully submitted,

Date: 22 January 2002

Anita L. Meiklejohn, Ph.D.
Reg. No. 35,283

Fish & Richardson P.C.
225 Franklin Street
Boston, Massachusetts 02110-2804
Telephone: (617) 542-5070
Facsimile: (617) 542-8906

Version with markings to show changes made

In the specification:

Paragraph beginning at page 1, line 8, has been amended as follos:

This application is a divisional of application serial number 09/099,041, filed June 17, 1998, which is a continuation-in-part of application serial number 09/019,942, filed February 6, 1998.

Paragraph beginning at page 3, line 24, has been amended as follows:

Two forms of CARD-4 exist in the cell, a long form, CARD-4L, and a short form, CARD-4S. The cDNA of CARD-4L described below (SEQ ID NO:7) has a 2859 nucleotide open reading frame (nucleotides 245-3103 of SEQ ID NO:7; SEQ ID NO:9) which encodes a 953 amino acid protein (SEQ ID NO:8). CARD-4L protein possesses a CARD domain (amino acids 15-114 of SEQ ID NO:8; SEQ ID NO:10). CARD-4L is also predicted to have a nucleotide binding domain which extends from about amino acid 198 to about amino acid 397 of SEQ ID NO:8; SEQ ID NO:11, a Walker Box "A", which extends from about amino acid 202 to about amino acid 209 of SEQ ID NO:8; SEQ ID NO:12, a Walker Box "B", which extends from about amino acid 280 to about amino acid 284, of SEQ ID NO:8; SEQ ID NO:13, a kinase 3a subdomain, which extends from about amino acid 327 to about amino acid 338 of SEQ ID NO:8; SEQ ID NO:14, and ten Leucine-rich repeats which extend from about amino acid 674 to about amino acid 950 of SEQ ID NO:8. The first Leucine-rich repeat extends from about amino acid 674 to about amino acid 701 of SEQ ID NO:8; SEQ ID NO:15. The second Leucine-rich repeat extends from about amino acid 702 to about amino acid 727 of SEQ ID NO:8; SEQ ID NO:16. The third Leucine-rich repeat extends from about amino acid 728 to about amino acid 754 of SEQ ID NO:8; SEQ ID NO:17. The fourth Leucine-rich repeat extends from about amino acid 755 to about amino acid 782 of SEQ ID NO:8; SEQ ID NO:18. The fifth Leucine-rich repeat extends from about amino acid 783 to about amino acid 810 of SEQ ID NO:8; SEQ ID NO:19. The sixth Leucine-rich repeat extends from about amino acid 811 to about amino acid 838 of SEQ ID NO:8; SEQ ID NO:20. The seventh Leucine-rich repeat extends from about amino acid

839 to about amino acid 866 of SEQ ID NO:8; SEQ ID NO:21. The eighth Leucine-rich repeat extends from about amino acid 867 to about amino acid 894 of SEQ ID NO:8; SEQ ID NO:22. The ninth Leucine-rich repeat extends from about amino acid 895 to about amino acid 922 of SEQ ID NO:8; SEQ ID NO:23 and the tenth leucine-rich repeat extends from about amino acid 923 to about amino acid 950 of SEQ ID NO:8; SEQ ID NO:24.

Paragraph beginning at page 7, line 16, has been amended as follows:

The invention features a nucleic acid molecule which is at least 45% (or 55%, 65%, 75%, 85%, 95%, or 98%) identical to the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:7, SEQ ID NO:9, SEQ ID:25, SEQ ID NO:27, the nucleotide sequence of the cDNA insert of the plasmid deposited with ATCC as Accession Number (the "cDNA of ATCC [_____] 203037"), the nucleotide sequence of the cDNA insert of the plasmid deposited with ATCC as Accession Number (the "cDNA of ATCC[____] 203035"), the nucleotide sequence of the cDNA insert of the plasmid deposited with ATCC as Accession Number (the "cDNA of ATCC[____] 203036"), or a complement thereof.

Paragraph beginning at page 7, line 28, has been amended as follows:

The invention features a nucleic acid molecule which includes a fragment of at least 150 (300, 325, 350, 375, 400, 425, 450, 500, 550, 600, 650, 700, 800, 900, 1000, 1300, 1600 or 1931) nucleotides of the nucleotide sequence shown in SEQ ID NO:1, or SEQ ID NO:3, or the nucleotide sequence of the cDNA ATCC [_____] 203037, or a complement thereof.

Paragraph beginning at page 7, line 33, has been amended as follows:

The invention also features a nucleic acid molecule which includes a fragment of at least 150 (350, 400, 450, 500, 550, 600, 650, 700, 800, 900, 1000, 1300, 1600, 1900, 2100, 2400, 2700, 3000, or 3382) nucleotides of the nucleotide sequence shown in SEQ ID NO:7, or SEQ ID NO:9, or the nucleotide sequence of the cDNA ATCC [_____] 203035, or a complement thereof.

Paragraph beginning at page 8, line 6, has been amended as follows:

Also within the invention is a nucleic acid molecule which includes a fragment of at least 150 (350, 400, 450, 500, 550, 600, 650, 700, 800, 900, 1000, 1300, 1600, 1900, 2100, 2400, 2700, and 3080) nucleotides of the nucleotide sequence shown in SEQ ID NO:25, or SEQ ID NO:27, or the nucleotide sequence of the cDNA ATCC [_____] 203036, or a complement thereof.

Paragraph beginning at page 8, line 13, has been amended as follows:

The invention features a nucleic acid molecule which includes a nucleotide sequence encoding a protein having an amino acid sequence that is at least 45% (or 55%, 65%, 75%, 85%, 95%, or 98%) identical to the amino acid sequence of SEQ ID NO:2, SEQ ID NO:8, SEQ ID NO: 26 or the amino acid sequence encoded by the cDNA of ATCC [____] 203037, the amino acid sequence encoded by the cDNA of ATCC[____] 203035, or the amino acid sequence encoded by the cDNA of ATCC [____] 203036.

Paragraph beginning at page 8, line 21, has been amended as follows:

In a preferred embodiment, a CARD-3 nucleic acid molecule has the nucleotide sequence shown in SEQ ID NO:1, or SEQ ID NO:3, or the nucleotide sequence of the cDNA of ATCC [_____] 203037. In another preferred embodiment, a CARD-4L nucleic acid molecule has the nucleotide sequence shown in SEQ ID NO:7, or SEQ ID NO:9, or the nucleotide sequence of the cDNA of ATCC [_____] 203035. In yet another preferred embodiment, a CARD-4S nucleic acid molecule has the nucleotide sequence shown in SEQ ID NO:25, or SEQ ID NO:27, or the nucleotide sequence of the cDNA of ATCC [_____] 203036.

Paragraph beginning at page 8, line 31, has been amended as follows:

Also within the invention is a nucleic acid molecule which encodes a fragment of a polypeptide having the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:8 or SEQ ID NO:26, the fragment including at least 15 (25, 30, 50, 100, 150, 300, 400 or 540, 600, 700, 800, 953) contiguous amino acids of SEQ ID NO:2 or SEQ ID NO:8 or SEQ ID NO:26 or the polypeptide encoded by the cDNA of ATCC Accession Number [___] 203037, or the

polypeptide encoded by the cDNA of ATCC Accession Number [_____] 203035, or the polypeptide encoded by the cDNA of ATCC Accession Number [_____] 203036.

Paragraph beginning at page 9, line 7, has been amended as follows:

The invention includes a nucleic acid molecule which encodes a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2 or an amino acid sequence encoded by the cDNA of ATCC Accession Number [____] 203037, wherein the nucleic acid molecule hybridizes to a nucleic acid molecule comprising SEQ ID NO:1 or SEQ ID NO:3 under stringent conditions. The invention also includes a nucleic acid molecule which encodes a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:8 or an amino acid sequence encoded by the cDNA of ATCC Accession Number [____] 203035, wherein the nucleic acid molecule hybridizes to a nucleic acid molecule comprising SEQ ID NO:7 or SEQ ID NO:9 under stringent conditions.

Paragraph beginning at page 9, line 21, has been amended as follows:

The invention also includes a nucleic acid molecule which encodes a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:26 or an amino acid sequence encoded by the cDNA of ATCC Accession Number [____] 203036, wherein the nucleic acid molecule hybridizes to a nucleic acid molecule comprising SEQ ID NO:25 or SEQ ID NO:27 under stringent conditions.

Paragraph beginning at page 11, line 11, has been amended as follows:

Also within the invention are: an isolated CARD-3 protein which is encoded by a nucleic acid molecule having a nucleotide sequence that is at least about 65%, preferably 75%, 85%, or 95% identical to SEQ ID NO:3 or the cDNA of ATCC [_____] 203037; an isolated CARD-3 protein which is encoded by a nucleic acid molecule having a nucleotide sequence at least about 65% preferably 75%, 85%, or 95% identical to the kinase domain encoding portion of SEQ ID NO:1 (e.g., about nucleotides 213 to 1113 of SEQ ID NO:1); an isolated CARD-3 protein which is encoded by a nucleic acid molecule having a nucleotide sequence at least about 65% preferably 75%, 85%, or 95% identical the linker domain encoding portion of SEQ ID NO:1

(e.g., about nucleotides 1114 to 1506 of SEQ ID NO:1); and an isolated CARD-3 protein which is encoded by a nucleic acid molecule having a nucleotide sequence at least about 65% preferably 75%, 85%, or 95% identical the CARD domain encoding portion of SEQ ID NO:1 (e.g., about nucleotides 1507 to 1833 of SEQ ID NO:1); and an isolated CARD-3 protein which is encoded by a nucleic acid molecule having a nucleotide sequence which hybridizes under stringent hybridization conditions to a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:3 or the non-coding strand of the cDNA of ATCC[_____] 203037.

Paragraph beginning at page 11, line 34, has been amended as follows:

Also within the invention are: an isolated CARD-4L protein which is encoded by a nucleic acid molecule having a nucleotide sequence that is at least about 65%, preferably 75%, 85%, or 95% identical to SEQ ID NO:9 or the cDNA of ATCC [_____] 203035; an isolated CARD-4L protein which is encoded by a nucleic acid molecule having a nucleotide sequence at least about 65% preferably 75%, 85%, or 95% identical the CARD domain encoding portion of SEQ ID NO:7 (e.g., about nucleotides 287 to 586 of SEQ ID NO:7); an isolated CARD-4L protein which is encoded by a nucleic acid molecule having a nucleotide sequence at least about 65% preferably 75%, 85%, or 95% identical the nucleotide binding domain encoding portion of SEQ ID NO:7 (e.g., about nucleotides 836 to 1436 of SEQ ID NO:7); an isolated CARD-4L protein which is encoded by a nucleic acid molecule having a kinase 3a subdomain at least about 65% preferably 75%, 85%, or 95% identical the nucleotide binding domain encoding portion of SEQ ID NO:7 (e.g., about nucleotides 1223 to 1258 of SEQ ID NO:7); an isolated CARD-4L protein which is encoded by a nucleic acid molecule having a nucleotide sequence at least about 65% preferably 75%, 85%, or 95% identical the Leucine-rich repeats encoding portion of SEQ ID NO:7 (e.g., about nucleotides 2264 to 2347 of SEQ ID NO:7; about nucleotides 2348 to 2425 of SEQ ID NO:7; about nucleotides 2426 to 2506 of SEQ ID NO:7; about nucleotides 2507 to 2590 of SEQ ID NO:7; about nucleotides 2591 to 2674 of SEQ ID NO:7; about nucleotides 2675 to 2758 of SEQ ID NO:7; about nucleotides 2759 to 2842 of SEQ ID NO:7; about nucleotides 2843 to 2926 of SEQ ID NO:7; about nucleotides 2927 to 3010 of SEQ ID NO:7; about nucleotides 3011 to 3094 of SEQ ID NO:7; and an isolated CARD-4L protein which is encoded by a nucleic acid molecule having a nucleotide sequence which hybridizes under stringent

hybridization conditions to a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:9 or the non-coding strand of the cDNA of ATCC [_____] 203035.

Paragraph beginning at page 13, line 1, has been amended as follows:

Also within the invention are: an isolated CARD-4S protein which is encoded by a nucleic acid molecule having a nucleotide sequence that is at least about 65%, preferably 75%, 85%, or 95% identical to SEQ ID NO:27 or the cDNA of ATCC [_____] 203036; an isolated CARD-3 protein which is encoded by a nucleic acid molecule having a nucleotide sequence at least about 65% preferably 75%, 85%, or 95% identical the CARD domain encoding portion of SEQ ID NO:25 (e.g., about nucleotides 1 to 222 of SEQ ID NO:25); an isolated CARD-3 protein which is encoded by a nucleic acid molecule having a nucleotide sequence at least about 65% preferably 75%, 85%, or 95% identical the P-Loop encoding portion of SEQ ID NO:25 (e.g., about nucleotides 485 to 510 of SEQ ID NO:25).

Paragraph beginning at page 13, line 14, has been amended as follows:

Also within the invention is a polypeptide which is a naturally occurring allelic variant of a polypeptide that includes the amino acid sequence of SEQ ID NO:2 or an amino acid sequence encoded by the cDNA insert of the plasmid deposited with ATCC as Accession Number [_____] 203037, wherein the polypeptide is encoded by a nucleic acid molecule which hybridizes to a nucleic acid molecule comprising SEQ ID NO:1 or SEQ ID NO:3 under stringent conditions.

Paragraph beginning at page 13, line 22, has been amended as follows:

Also within the invention is a polypeptide which is a naturally occurring allelic variant of a polypeptide that includes the amino acid sequence of SEQ ID NO:8 or an amino acid sequence encoded by the cDNA insert of the plasmid deposited with ATCC as Accession Number [_____] 203035, wherein the polypeptide is encoded by a nucleic acid molecule which hybridizes to a nucleic acid molecule comprising SEQ ID NO:7 or SEQ ID NO:9 under stringent conditions.

Paragraph beginning at page 13, line 30, has been amended as follows:

Also within the invention is a polypeptide which is a naturally occurring allelic variant of a polypeptide that includes the amino acid sequence of SEQ ID NO:26 or an amino acid sequence encoded by the cDNA insert of the plasmid deposited with ATCC as Accession Number [_____] 203036, wherein the polypeptide is encoded by a nucleic acid molecule which hybridizes to a nucleic acid molecule comprising SEQ ID NO:25 or SEQ ID NO:27 under stringent conditions.

Paragraph beginning at page 14, line 4, has been amended as follows:

Another embodiment of the invention features CARD-3 or CARD-4L/S nucleic acid molecules which specifically detect CARD-3 or CARD-4L/S nucleic acid molecules, relative to nucleic acid molecules encoding other members of the CARD superfamily. For example, in one embodiment, a CARD-4L nucleic acid molecule hybridizes under stringent conditions to a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:7, SEQ ID NO:9, or the cDNA of ATCC [_____] 203035, or a complement thereof. In another embodiment, the CARD-4L nucleic acid molecule is at least 300 (350, 400, 450, 500, 550, 600, 650, 700, 800, 900, 1000, 1300, 1600, 1900, 2100, 2400, 2700, 3000, or 3382) nucleotides in length and hybridizes under stringent conditions to a nucleic acid molecule comprising the nucleotide sequence shown in SEQ ID NO:7, SEQ ID NO:9, the cDNA of ATCC [_____] 203035, or a complement thereof. In another embodiment, an isolated CARD-4L nucleic acid molecule comprises nucleotides 287 to 586 of SEQ ID NO:7, encoding the CARD domain of CARD-4L, or a complement thereof. In yet another embodiment, the invention provides an isolated nucleic acid molecule which is antisense to the coding strand of a CARD-4L nucleic acid.

Paragraph beginning at page 17, line 21, has been amended as follows:

Figures 3A-3B depict[s] the cDNA sequence (SEQ ID NO:7) of CARD-4L. The open reading frame of SEQ ID NO:7 extends from nucleotide 245 to nucleotide 3103 (SEQ ID NO:9).

Paragraph beginning at page 17, line 26, has been amended as follows:

Figure 5 depicts the partial cDNA sequence (SEQ ID NO:25) of CARD-4S [and the predicted amino acid sequence (SEQ ID NO:25) of human CARD-4S]. The open reading frame of CARD-4S (SEQ ID NO:25) extends from nucleotide 1 to nucleotide 1470 (SEQ ID NO:27).

Paragraph beginning at page 17, line 33, has been amended as follows:

Figure 7 depicts an alignment of the CARD domains of CARD-4 (SEQ ID NO:10), CARD-3 (SEQ ID NO:6), ARC-CARD (SEQ ID NO:31), cIAP1-CARD (SEQ ID NO:32), [cIAP1-CARD (SEQ ID NO:33)] and cIAP2-CARD (SEQ ID NO:[34]33).

Paragraph beginning at page 18, line 9, has been amended as follows:

The present invention is based on the discovery of a cDNA molecule encoding human CARD-3 and human CARD-4 proteins. A nucleotide sequence encoding a human CARD-3 protein is shown in Figure 1 (SEQ ID NO:1; SEQ ID NO:3 includes the open reading frame only). A predicted amino acid sequence of CARD-3 protein is also shown in Figure 2 (SEQ ID NO: 2). CARD-4 has two forms, a long form, CARD-4L, and a short form, CARD-4S. A nucleotide sequence encoding a human CARD-4L protein is shown in Figures 3A-3B (SEQ ID NO:7; SEQ ID NO:9 includes the open reading frame only). A predicted amino acid sequence of CARD-4L protein is also shown in Figure 4 (SEQ ID NO:8). A nucleotide sequence encoding a partial human CARD-4S protein is shown in Figure 5 (SEQ ID NO:25; SEQ ID NO:27 includes the open reading frame only). A predicted amino acid sequence of CARD-4S protein is also shown in Figure 6 (SEQ ID NO:26).

Paragraph beginning at page 18, line 25, has been amended as follows:

The human CARD-3 cDNA of Figure 1 (SEQ ID NO:1), which is approximately 1931 nucleotides long including untranslated regions, encodes a protein amino acid having a molecular weight of approximately 61 kDa (excluding post-translational modifications). A plasmid containing a cDNA encoding human CARD-3 [(with the cDNA insert name of ____)] (pXE17A) was deposited with the American Type Culture Collection (ATCC), [Rockville, Maryland] 10801 University Boulevard, Manassas, VA on [____] May 14, 1998, and assigned

Accession Number [___] 203037. This deposit will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. This deposit was made merely as a convenience for those of skill in the art and is not an admission that a deposit is required under 35 U.S.C. ' 112.

Paragraph beginning at page 19, line 5, has been amended as follows:

The human CARD-4L cDNA of Figures 3A-3B (SEQ ID NO:7), which is approximately 3382 nucleotides long including untranslated regions, encodes a protein amino acid having a molecular weight of approximately 108 kDa (excluding post-translational modifications). A plasmid containing a cDNA encoding human CARD-4L [(with the cDNA insert name of ___)] (pC4L1) was deposited with the American Type Culture Collection (ATCC), [Rockville, Maryland] 1801 University Boulevard, Manassas, VA on [___] May 14, 1998, and assigned Accession Number [___] 203035. This deposit will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. This deposit was made merely as a convenience for those of skill in the art and is not an admission that a deposit is required under 35 U.S.C. 112.

Paragraph beginning at page 19, line 19, has been amended as follows:

The human partial CARD-4S cDNA of Figure 5 (SEQ ID NO:25), which is approximately 3080 nucleotides long including untranslated regions. A plasmid containing a cDNA encoding human CARD-4S [(with the cDNA insert name of ___)] (pDB33E) was deposited with the American Type Culture Collection (ATCC), [Rockville, Maryland] 1801 University Boulevard, Manassas, VA on [___] May 14, 1998, and assigned Accession Number [___] 203036. This deposit will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. This deposit was made merely as a convenience for those of skill in the art and is not an admission that a deposit is required under 35 U.S.C. §112.

Paragraph beginning at page 19, line 31, has been amended as follows:

A region of human CARD-4L protein (SEQ ID NO:8) bears some similarity to a CARD domain of CARD-3 (SEQ ID NO:6), ARC-CARD (SEQ ID NO:31), cIAP1-CARD (SEQ ID NO:32), [cIAP1-CARD (SEQ ID NO:33)] and cIAP2-CARD (SEQ ID NO:[34]33). This comparison is depicted in Figure 7.

Paragraph beginning at page 20, line 22, has been amended as follows:

Preferred CARD-3 or CARD-4L or CARD-4S polypeptides of the present invention have an amino acid sequence sufficiently identical to the CARD domain consensus amino acid sequence of SEQ ID NO:6, SEQ ID NO:10, SEQ ID NO:28, respectively. The CARD-3 polypeptide also has an amino acid sequence sufficiently identical to the kinase domain consensus sequence of SEQ ID NO:4, and an amino acid sequence that is sufficiently identical to the linker domain of SEQ ID NO:5. The CARD-4L polypeptide has an amino acid sequence sufficiently identical to the nucleotide binding domain of SEQ ID NO:11, an amino acid sequence sufficiently identical to the Walker Box "A" of SEQ ID NO:12 or Walker Box "B" of SEQ ID NO:13, or an amino acid sequence sufficiently identical to the kinase 3a subdomain of SEQ ID NO:14, or an amino acid sequence sufficiently identical to the Leucine-rich repeats of [SEQ ID NO:14], SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO: 19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO: 22, [and] SEQ ID NO: 23, and SEQ ID NO:24. As used herein, the term "sufficiently identical" refers to a first amino acid or nucleotide sequence which contains a sufficient or minimum number of identical or equivalent (e.g., an amino acid residue which has a similar side chain) amino acid residues or nucleotides to a second amino acid or nucleotide sequence such that the first and second amino acid or nucleotide sequences have a common structural domain and/or common functional activity. For example, amino acid or nucleotide sequences which contain a common structural domain having about 65% identity, preferably 75% identity, more preferably 85%, 95%, or 98% identity are defined herein as sufficiently identical.

Paragraph beginning at page 23, line 7, has been amended as follows:

A nucleic acid molecule of the present invention, e.g., a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO: 25, SEQ ID NO: [26]27, the cDNA of ATCC [_____] 203037, the cDNA of ATCC [_____] 203035, or the cDNA of ATCC [_____] 203036, or a complement of any of these nucleotide sequences, can be isolated using standard molecular biology techniques and the sequence information provided herein. Using all or portion of the nucleic acid sequences of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO: 25, SEQ ID NO: [26]27, the cDNA of ATCC [_____] 203037, the cDNA of ATCC [____] 203035, or the cDNA of ATCC [____] 203036 as a hybridization probe, CARD-3 or CARD-4L/S nucleic acid molecules can be isolated using standard hybridization and cloning techniques (e.g., as described in Sambrook et al., eds., *Molecular Cloning: A Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory,* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989).

Paragraph beginning at page 23, line 33, has been amended as follows:

In another preferred embodiment, an isolated nucleic acid molecule of the invention comprises a nucleic acid molecule which is a complement of the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO: 25, SEQ ID NO: [26]27, the cDNA of ATCC [_____] 203037, the cDNA of ATCC [_____] 203035, the cDNA of ATCC [_____] 203036, or a portion thereof. A nucleic acid molecule which is complementary to a given nucleotide sequence is one which is sufficiently complementary to the given nucleotide sequence that it can hybridize to the given nucleotide sequence thereby forming a stable duplex.

Paragraph beginning at page 24, line 10, has been amended as follows:

Moreover, the nucleic acid molecule of the invention can comprise only a portion of a nucleic acid sequence encoding CARD-3 or CARD-4L/S, for example, a fragment which can be used as a probe or primer or a fragment encoding a biologically active portion of CARD-3 or CARD-4L/S. The nucleotide sequence determined from the cloning of the human CARD-3 or CARD-4L/S gene allows for the generation of probes and primers designed for use in identifying

and/or cloning CARD-3 or CARD-4L/S homologues in other cell types, e.g., from other tissues, as well as CARD-3 or CARD-4L/S homologues from other mammals. The probe/primer typically comprises substantially purified oligonucleotide. The oligonucleotide typically comprises a region of nucleotide sequence that hybridizes under stringent conditions to at least about 12, preferably about 25, more preferably about 50, 75, 100, 125, 150, 175, 200, 250, 300, 350 or 400 consecutive nucleotides of the sense or anti-sense sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:7, SEQ ID NO:9, the cDNA of ATCC [_____] 203037, the cDNA of ATCC [_____] 203035, or of a naturally occurring mutant of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:7, SEQ ID NO:9, the cDNA of ATCC [_____] 203037, or the cDNA of ATCC [_____] 203035.

Paragraph beginning at page 25, line 11, has been amended as follows:

A nucleic acid fragment encoding a "biologically active portion of CARD-3 or CARD-4L/S" can be prepared by isolating a portion of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:7, SEQ ID NO:9, or the nucleotide sequence of the cDNA of ATCC [_____] 203037, or the nucleotide sequence of the cDNA of ATCC [_____] 203035 which encodes a polypeptide having a CARD-3 or CARD-4L/S biological activity, expressing the encoded portion of CARD-3 or CARD-4L/S protein (e.g., by recombinant expression *in vitro*) and assessing the activity of the encoded portion of CARD-3 or CARD-4L/S. For example, a nucleic acid fragment encoding a biologically active portion of CARD-3 or CARD-4L/S includes a CARD domain, e.g., SEQ ID NO:6 and SEQ ID NO:10 or SEQ ID NO:[26]28.

Paragraph beginning at page 25, line 24, has been amended as follows:

The invention further encompasses nucleic acid molecules that differ from the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:25, SEQ ID NO:27, the cDNA of ATCC [_____] 203037, or the cDNA of ATCC [_____] 203035, or the cDNA of ATCC [_____] 203036 due to degeneracy of the genetic code and thus encode the same CARD-3 or CARD-4L/S protein as that encoded by the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:25, SEQ ID NO:27, the

cDNA of ATCC [_____] 203037, the cDNA of ATCC [_____] 203035, or the cDNA of ATCC [_____] 203036.

Paragraph beginning at page 25, line 34, has been amended as follows:

In addition to the human CARD-3 or CARD-4L/S nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:25, SEQ ID NO:27, the cDNA of ATCC [_____] 203037, the cDNA of ATCC [_____] 203035, or the cDNA of ATCC [_____] 203036, it will be appreciated by those skilled in the art that DNA sequence polymorphisms that lead to changes in the amino acid sequences of CARD-3 or CARD-4L/S may exist within a population (e.g., the human population). Such genetic polymorphism in the CARD-3 or CARD-4L/S gene may exist among individuals within a population due to natural allelic variation. As used herein, the terms "gene" and "recombinant gene" refer to nucleic acid molecules comprising an open reading frame encoding a CARD-3 or CARD-4L/S protein, preferably a mammalian CARD-3 or CARD-4L/S protein. Such natural allelic variations can typically result in 1-5% variance in the nucleotide sequence of the CARD-3 or CARD-4L/S gene. Any and all such nucleotide variations and resulting amino acid polymorphisms in CARD-3 or CARD-4L/S that are the result of natural allelic variation and that do not alter the functional activity of CARD-3 or CARD-4L/S are intended to be within the scope of the invention.

Paragraph beginning at page 26, line 34, has been amended as follows:

Accordingly, in another embodiment, an isolated nucleic acid molecule of the invention is at least 300 (325, 350, 375, 400, 425, 450, 500, 550, 600, 650, 700, 800, 900, 1000, 1300, 1600 or 1931) nucleotides in length and hybridizes under stringent conditions to the nucleic acid molecule comprising the nucleotide sequence, preferably the coding sequence, of SEQ ID NO:1, SEQ ID NO:3, or the cDNA of ATCC [_____] 203037. In yet another embodiment, an isolated nucleic acid molecule of the invention is at least 300 (325, 350, 375, 400, 425, 450, 500, 550, 600, 650, 700, 800, 900, 1000, or 1300, 1640, 1900, 2200, 2500, 2800, 3100, or 3382) nucleotides in length and hybridizes under stringent conditions to the nucleic acid molecule comprising the nucleotide sequence, preferably the coding sequence, of SEQ ID NO:7, SEQ ID NO:9, the cDNA of ATCC [_____] 203035.

Paragraph beginning at page 27, line 15, has been amended as follows:

As used herein, the term "hybridizes under stringent conditions" is intended to describe conditions for hybridization and washing under which nucleotide sequences at least 60% (65%, 70%, preferably 75%) identical to each other typically remain hybridized to each other. Such stringent conditions are known to those skilled in the art and can be found in *Current Protocols in Molecular Biology*, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. A preferred, non-limiting example of stringent hybridization conditions are hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45EC, followed by one or more washes in 0.2 X SSC, 0.1% SDS at 50-65EC. Preferably, an isolated nucleic acid molecule of the invention that hybridizes under stringent conditions to the sequence of SEQ ID NO:1, SEQ ID NO:3, the cDNA of ATCC [_____] 203037 corresponds to a naturally-occurring nucleic acid molecule. As used herein, a "naturally-occurring" nucleic acid molecule refers to an RNA or DNA molecule having a nucleotide sequence that occurs in nature (e.g., encodes a natural protein).

Paragraph beginning at page 27, line 34, has been amended as follows:

In addition to naturally-occurring allelic variants of the CARD-3 or CARD-4L/S sequence that may exist in the population, the skilled artisan will further appreciate that changes can be introduced by mutation into the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:25, SEQ ID NO:[26]27, the cDNA of ATCC [_____] 203037, the cDNA of ATCC [_____] 203035, or the cDNA of ATCC [____] 203036, thereby leading to changes in the amino acid sequence of the encoded CARD-3 or CARD-4L/S protein, without altering the functional ability of the CARD-3 or CARD-4L/S protein. For example, one can make nucleotide substitutions leading to amino acid substitutions at "non-essential" amino acid residues. A "non-essential" amino acid residue is a residue that can be altered from the wild-type sequence of CARD-3 or CARD-4L/S (e.g., the sequence of SEQ ID NO:2 or SEQ ID NO:8 or SEQ ID NO:26) without altering the biological activity, whereas an "essential" amino acid residue is required for biological activity. For example, amino acid residues that are

conserved among the CARD-3 or CARD-4L/S proteins of various species are predicted to be particularly unamenable to alteration.

Paragraph beginning at page 28, line 32, has been amended as follows:

Accordingly, another aspect of the invention pertains to nucleic acid molecules encoding CARD-3 or CARD-4L/S proteins that contain changes in amino acid residues that are not essential for activity. Such CARD-3 or CARD-4L/S proteins differ in amino acid sequence from SEQ ID NO:2 or SEQ ID NO: 8 or SEQ ID NO:[25]26 and yet retain biological activity. In one embodiment, the isolated nucleic acid molecule includes a nucleotide sequence encoding a protein that includes an amino acid sequence that is at least about 45% identical, 65%, 75%, 85%, 95%, or 98% identical to the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:8 or SEQ ID NO:26.

Paragraph beginning at page 29, line 10, has been amended as follows:

An isolated nucleic acid molecule encoding a CARD-3 or CARD-4L/S protein having a sequence which differs from that of SEQ ID NO:2 or SEQ ID NO:8 or SEQ ID NO:26, respectively, can be created by introducing one or more nucleotide substitutions, additions or deletions into the nucleotide sequence of CARD-3 (SEQ ID NO:1, SEQ ID NO:3, the cDNA of ATCC [_____] 203037) or CARD-4L (SEQ ID NO:7, SEQ ID NO:9, the cDNA of ATCC [____] 203035), or CARD-4S (SEQ ID NO:25, SEQ ID NO:27, the cDNA of ATCC [____] 203036) such that one or more amino acid substitutions, additions or deletions are introduced into the encoded protein. Mutations can be introduced by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis. Preferably, conservative amino acid substitutions are made at one or more predicted non-essential amino acid residues. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine,

methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Thus, a predicted nonessential amino acid residue in CARD-3 or CARD-4L/S is preferably replaced with another amino acid residue from the same side chain family. Alternatively, mutations can be introduced randomly along all or part of a CARD-3 or CARD-4L/S coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for CARD-3 or CARD-4L/S biological activity to identify mutants that retain activity. Following mutagenesis, the encoded protein can be expressed recombinantly and the activity of the protein can be determined.

Paragraph beginning at page 31, line 4, has been amended as follows:

Given the coding strand sequences encoding CARD-3 or CARD-4L/S disclosed herein (e.g., SEQ ID NO:1 or SEQ ID NO:3 or SEQ ID NO:7 or SEQ ID NO:9 or SEQ ID NO:25 or SEQ ID NO:27), antisense nucleic acids of the invention can be designed according to the rules of Watson and Crick base pairing. The antisense nucleic acid molecule can be complementary to the entire coding region of CARD-3 or CARD-4L/S mRNA, but more preferably is an oligonucleotide which is antisense to only a portion of the coding or noncoding region of CARD-3 or CARD-4L/S mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding the translation start site of CARD-3 mRNA, e.g., an oligonucleotide having the sequence CCCTGGTACTTGCCCCTCCGGTAG (SEQ ID NO:[35]34) or CCTGGTACTTGCCCCTCC (SEQ ID NO:[36]35) or of the CARD-4L mRNA e.g., TCGTTAAGCCCTTGAAGACAGTG (SEQ ID NO:[37]36) and TCGTTAGCCCTTGAAGACCAGTGAGTGTAG (SEQ ID NO:[38]37). An antisense oligonucleotide can be, for example, about 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 nucleotides in length. An antisense nucleic acid of the invention can be constructed using chemical synthesis and enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid (e.g., an antisense oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, e.g., phosphorothioate derivatives and acridine substituted nucleotides can be used. Examples of modified nucleotides which can be used to generate the

antisense nucleic acid include 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine. Alternatively, the antisense nucleic acid can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (i.e., RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest, described further in the following subsection).

Paragraph beginning at page 38, line 9, has been amended as follows:

Biologically active portions of a CARD-3 or CARD-4L/S protein include peptides comprising amino acid sequences sufficiently identical to or derived from the amino acid sequence of the CARD-3 or CARD-4L/S protein (e.g., the amino acid sequence shown in SEQ ID NO:2 or SEQ ID NO:8 or SEQ ID NO:26), which include less amino acids than the full length CARD-3 or CARD-4L/S proteins, and exhibit at least one activity of a CARD-3 or CARD-4L/S protein. Typically, biologically active portions comprise a domain or motif with at least one activity of the CARD-3 or CARD-4L/S protein. A biologically active portion of a CARD-3 or CARD-4L/S protein can be a polypeptide which is, for example, 10, 25, 50, 100 or more amino acids in length. Preferred biologically active polypeptides include one or more identified CARD-3 or CARD-4L/S structural domains, e.g., the CARD domain (SEQ ID NO:6 or SEQ ID NO:10 or SEQ ID NO:[27]<u>28</u>).

Paragraph beginning at page 38, line 30, has been amended as follows:

Preferred CARD-3 or CARD-4L/S protein has the amino acid sequence shown of SEQ ID NO:2 or SEQ ID NO:8 or SEQ ID NO:26, respectively. Other useful CARD-3 or CARD-4L/S proteins are substantially identical to SEQ ID NO:2 or SEQ ID NO:8 or SEQ ID NO:26, respectively, and retain the functional activity of the protein of SEQ ID NO:2 or SEQ ID NO:8 or SEQ ID NO:26, yet differ in amino acid sequence due to natural allelic variation or mutagenesis. CARD-3 and CARD-4L/S may be involved in activating caspases in the apoptotic pathway. Accordingly, a useful CARD-3 or CARD-4L/S protein is a protein which includes an amino acid sequence at least about 45%, preferably 55%, 65%, 75%, 85%, 95%, or 99% identical to the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:8 or SEQ ID NO:26 and retains the functional activity of the CARD-3 or CARD-4L/S proteins of SEQ ID NO:2 or SEQ ID NO:8 or SEQ ID NO:26. In other instances, the CARD-3 or CARD-4L/S protein is a protein having an amino acid sequence 55%, 65%, 75%, 85%, 95%, or 98% identical to the CARD-3 or CARD-4L CARD domain (SEQ ID NO:6, SEQ ID NO:10 and SEQ ID NO:[27]28). In a preferred embodiment, the CARD-3 or CARD-4L/S protein retains a functional activity of the CARD-3 or CARD-4L/S protein of SEQ ID NO:2, SEQ ID NO:8 or SEQ ID NO:26.

Paragraph beginning at page 60, line 6, has been amended as follows:

A transgenic animal of the invention can be created by introducing CARD-3 or CARD-4L/S-encoding nucleic acid into the male pronuclei of a fertilized oocyte, e.g., by microinjection, retroviral infection, and allowing the oocyte to develop in a pseudopregnant female foster animal. The CARD-3 or CARD-4L/S cDNA sequence e.g., that of (SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:25, SEQ ID NO:27, or the cDNA of ATCC [_____] 203037, or the cDNA of ATCC [_____] 203035, or the cDNA of ATCC [_____] 203036) can be introduced as a transgene into the genome of a non-human animal. Alternatively, a nonhuman homologue of the human CARD-3 or CARD-4L/S gene, such as a mouse CARD-3 or CARD-4L/S gene, can be isolated based on hybridization to the human CARD-3 or CARD-4L/S cDNA and used as a transgene. Intronic sequences and polyadenylation signals can also be included in the transgene to increase the efficiency of expression of the transgene. A tissue-specific regulatory sequence(s) can be operably linked to

the CARD-3 or CARD-4L/S transgene to direct expression of CARD-3 or CARD-4L/S protein to particular cells. Methods for generating transgenic animals via embryo manipulation and microinjection, particularly animals such as mice, have become conventional in the art and are described, for example, in U.S. Patent Nos. 4,736,866 and 4,870,009, U.S. Patent No. 4,873,191 and in Hogan, *Manipulating the Mouse Embryo*, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1986). Similar methods are used for production of other transgenic animals.

Paragraph beginning at page 104, line 9, has been amended as follows:

The human CARD-4L cDNA was isolated as described above (Figures 3A-3B; SEQ ID NO:7) and has a 2859 nucleotide open reading frame (nucleotides 245-3103 of SEQ ID NO:7; SEQ ID NO:9) which encodes a 953 amino acid protein (Figure 4;SEQ ID NO:8). CARD-4L protein has a predicted CARD domain (amino acids 15-114; SEQ ID NO:10). CARD-4L is also predicted to have a nucleotide binding domain which extends from about amino acid 198 to about amino acid 397 of SEQ ID NO:8; SEQ ID NO:11, a predicted Walker Box "A", which extends from about amino acid 202 to about amino acid 209 of SEQ ID NO:8; SEQ ID NO:12, a predicted Walker Box "B", which extends from about amino acid 280 to about amino acid 284, of SEQ ID NO:8; SEQ ID NO:13, a predicted kinase 3a subdomain, which extends from about amino acid 327 to about amino acid 338 of SEQ ID NO:8; SEQ ID NO:14, and ten predicted Leucine-rich repeats which extend from about amino acid 674 to about amino acid 950 of SEQ ID NO:8. The first Leucine-rich repeat is predicted to extend from about amino acid 674 to about amino acid 701 of SEQ ID NO:8; SEQ ID NO:15. The second Leucine-rich repeat is predicted to extend from about amino acid 702 to about amino acid 727 of SEQ ID NO:8; SEQ ID NO:16. The third Leucine-rich repeat is predicted to extend from about amino acid 728 to about amino acid 754 of SEQ ID NO:8; SEQ ID NO:17. The fourth Leucine-rich repeat is predicted to extend from about amino acid 755 to about amino acid 782 of SEQ ID NO:8; SEQ ID NO:18. The fifth Leucine-rich repeat is predicted to extend from about amino acid 783 to about amino acid 810 of SEQ ID NO:8; SEQ ID NO:19. The sixth Leucine-rich repeat is predicted to extend from about amino acid 811 to about amino acid 838 of SEQ ID NO:8; SEQ ID NO:20. The seventh Leucine-rich repeat is predicted to extend from about amino acid 839 to

about amino acid 866 of SEQ ID NO:8; SEQ ID NO:21. The eighth Leucine-rich repeat is predicted to extend from about amino acid 867 to about amino acid 894 of SEQ ID NO:8; SEQ ID NO:22. The ninth Leucine-rich repeat is predicted to extend from about amino acid 895 to about amino acid 922 of SEQ ID NO:8; SEQ ID NO:23 and the tenth leucine-rich repeat is predicted to extend from about amino acid 923 to about amino acid 950 of SEQ ID NO:8; SEQ ID NO:24.

In the claims:

Claims 1-23 have been cancelled.